HAEMOGLOBIN POLYMORPHISM AND GENETIC IDENTITIES IN FIVE INDIAN COMMENSAL RODENT SPECIES[1]

M. S. PRADHAN[2], A. M. BHAGWAT[3] AND S. T. INGALE[4]

(With two text-figures)

PAGE was used to study haemoglobin polymorphism in five Indian rodent species from Bombay-Pune region. *Rattus rattus rufescens* (Gray) showed the maximum number (15) of bands, whereas *Rattus r. wroughtoni* Hinton had only seven bands. Genetic identities pointed towards the closeness of *Rattus norvegicus* (Berkenhout) and *Bandicota bengalensis kok* (Gray). Maximum genetic distance was between *R. r. rufescens* and *R. r. wroughtoni*. The results support the earlier proposal of elevation of *R. r. rufescens* to a separate species.

INTRODUCTION

Biochemical differentiation of species specific proteins could be established by using electrophoretic techniques (Selander *et al.* 1969, Yoshida *et al.* 1971, De Smet and William 1978). We have already recorded differences in electrophoretic mobilities of haemoglobin on paper for Indian commensal rodents from Bombay-Poona region (Pradhan 1982, Pradhan *et al.* 1985); a polymorphic pattern for the inheritance of haemoglobin in rodents was established. It was, therefore, felt that the use of PAGE to resolve the haemoglobin patterns in Indian rodents may bring out additional information regarding haemoglobin polymorphism, frequency occurrence of polymorphic locii and possible genetic inter-relations. For the present study five predominantly commensal species belonging to two rodent genera, namely *Bandicota* and *Rattus*, were selected.

MATERIAL AND METHODS

About 91 specimens belonging to the five commensal rodent species were collected from Bombay-Pune region, Maharashtra, with the help of municipal workers. The specimens were killed for blood sample collection. The haemoglobin was separated as per the methods described by Wright (1974) while the PAGE was carried out by the method given by Zweig and Whitaker (1967) using 7.5% gels in Tris-HCL buffer. Tris-Glycin (pH 8.5) was used as electrode buffer. All the bands obtained were recorded and their mobilities were calculated in relation to that of the marker Bromophenol blue (RF values). From such individual records a common pattern for the species was evolved. The genetic identities (I) and genetic distances (D) were calculated with the help of Nei (1972). The dendrogram for the five species was constructed by unweighted pair-group arithmetic average (UPGMA) cluster analysis method (Sneath and Sokal 1973). The identification of each specimen was carried out with the help of Ellerman (1961) at Zoological Survey of India, Western Regional Station, Pune, while the PAGE was carried out at R. J. College, Ghatkopar, Bombay.

RESULTS AND DISCUSSION

In the preliminary analysis on PAGE, haemoglobin, collected from every specimen of the five selected species, was subjected to electrophoretic separation. All the bands obtained were recorded and their mobilities were calculated in relation to that of the marker. Bromophenol blue (RF values). From such individual records a common pattern for the species was evolved. Table 1 records the relative mobilities of haemoglobin bands for the rodent species studied. The same data are represented

[1]Accepted October 1988.

[2]Zoological Survey of India, Western Regional Station, Pune- 411005

[3, 4]R. J. College, Ghatkopar, Bombay-400086.

as a consolidated diagrammatic haemoglobin profile for individual species (Fig. 1). Of the five different species studied *Rattus rattus rufescens* (Gray) appears to have the maximum haemoglobin polymorphism, with as many as 15 separate bands, and *R. rattus wroughtoni* Hinton the minimum, with a total of seven bands. Ferguson (1980) has suggested that the variations in the mobilities of protein (haemoglobin) bands may represent a polymorphism at gene locii regulating the synthesis of such proteins. Further, the haemoglobin molecule being a tetramer, modification at a single gene locus will give as many as five variants. It is generally accepted that among vertebrates, there are at least four gene locii regulating haemoglobin sysnthesis (Dobzansky *et al.* 1976, Fitch and Morgoliash 1970). On the basis of this assumption a total of as many as 22 bands may be expected for a haemoglobin molecule with a single alletic variation. The pattern of haemoglobin obtained in the present study also is in conformity with such an assumption.

Analysis of consolidated haemoglobin patterns for the five rodent species of two genera *Rattus* and *Bandicota* indicates that several bands have identical electrophoretic mobilities in all the species studied. This observation prompted us to calculate genetic identity (I) and genetic distance (D) (Table 2) based on allelic frequencies at each locus (Nei 1972). Based on

TABLE 1

HAEMOGLOBIN VARIANTS IN TERMS OF VALUES, THEIR OCCURRENCE AND FREQUENCIES IN THE POPULATIONS OF FIVE COMMENSAL RODENT SPECIES

Names of species	RF values with S.D.	No. of bands	% population (occurrence)	% frequency of bands
n=Total No. of specimens	0.1 ± —	2	6.1	2.6
nb=Total No. of bands	0.18 ± 0.02	3	9.1	3.8
Rattus rattus rufescens (Gray) n=33 nb=78	0.23 ± —	1	3.0	1.3
	0.3 ± —	2	6.1	2.6
	0.34 ± —	1	3.0	1.5
	0.38 ± —	3	9.1	3.8
	0.42 ± 0.01	4	12.1	5.1
	0.46 ± 0.02	14	42.4	17.9
	0.5 ± 0.01	8	24.2	10.3
	0.52 ± 0.01	4	12.1	5.1
	0.55 ± 0.01	10	30.3	12.8
	0.58 ± 0.01	9	27.3	11.5
	0.67 ± 0.01	13	39.4	16.6
	0.7 ± 0.01	3	9.1	3.8
	0.89 ± —	1	3.0	1.3
Rattus norvegicus (Berkenout) n=15 nb=46	0.21 ± 0.01	2	13.3	4.4
	0.25 ± 0.07	2	13.3	4.4
	0.30 ± 0.01	4	26.6	8.7
	0.34 ± 0.01	2	13.3	4.4
	0.38 ± 0.01	3	19.9	6.5
	0.45 ± 0.01	9	59.9	19.6
	0.49 ± 0.01	8	53.3	17.4
	0.53 ± 0.01	6	39.9	13.1
	0.59 ± 0.02	8	53.3	17.4
	0.65 ± —	2	13.3	4.4

TABLE 1 (contd.)

Names of species	RF values with S.D.	No. of bands	% population (occurrence)	% frequency of bands
Rattus rattus wroughtoni Hinton n=11 nb=22	0.4 ± —	1	9.1	4.6
	0.45 ± —	1	9.1	4.6
	0.5 ± 0.01	3	27.3	13.6
	0.53 ± 0.01	5	45.5	22.7
	0.59 ± 0.01	6	54.6	27.2
	0.63 ±0 .01	3	27.3	13.6
	0.66 ± 0.01	3	27.3	13.6
Bandicota indica indica (malabarica))(Bech.) n=13 nb=28	0.31 ± —	1	7.7	3.6
	0.36 ± 0.02	4	30.8	14.3
	0.42 ± 0.01	4	30.8	14.3
	0.46 ± 0.01	5	38.5	17.9
	0.49 ± 0.01	5	38.5	17.9
	0.53 ± 0.01	3	23.1	10.7
	0.57 ± 0.01	3	23.1	10.7
	0.62 ± 0.02	3	23.1	10.7
Bandicota bengalensis kok (lordi) (Gray) n=19 nb=44	0.32 ± —	1	5.3	2
	0.39 ± 0.01	5	26.3	10
	0.45 ± 0.01	8	42.1	16
	0.49 ± 0.01	7	36.8	14
	0.53 ± 0.01	1	57.9	22
	0.59 ± 0.01	7	36.8	14
	0.65 ± 0.01	4	21.4	8
	0.71 ± —	1	5.3	2

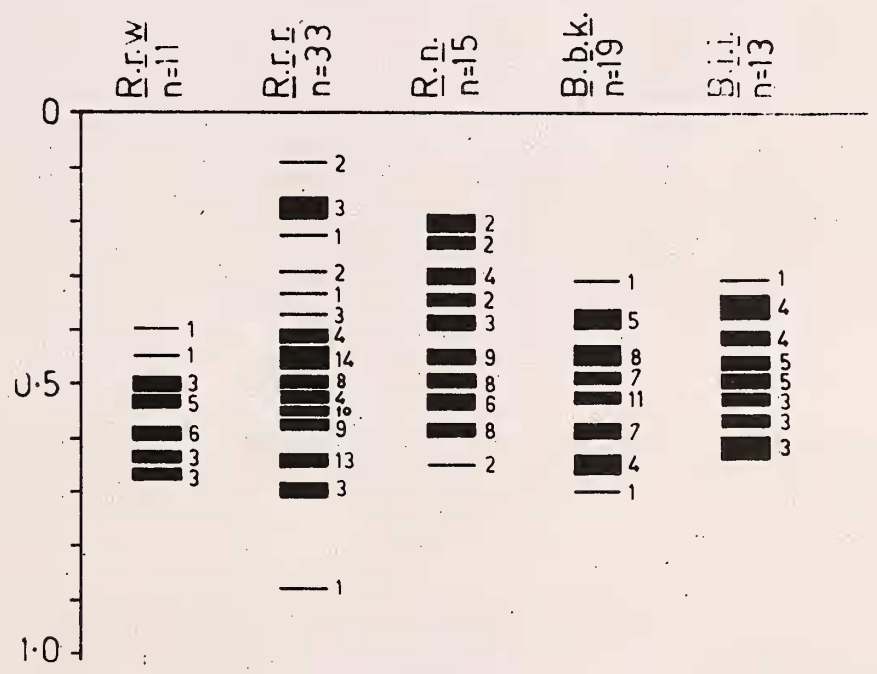

Fig. 1. Consolidated diagrammatic haemoglobin profile for individual species
R.r.w. = *Rattus rattus wroughtoni*, R.r.r. = *R. r. rufescens*, R.n. = *R. norvegicus*, B.b.k. = *Bandicota bengalensis kok (lordi)*, B.i.i. = *B. indica indica (malabarica)* , n = no. of specimens. Numbers near the bands show number of occurrences of each band in the population.

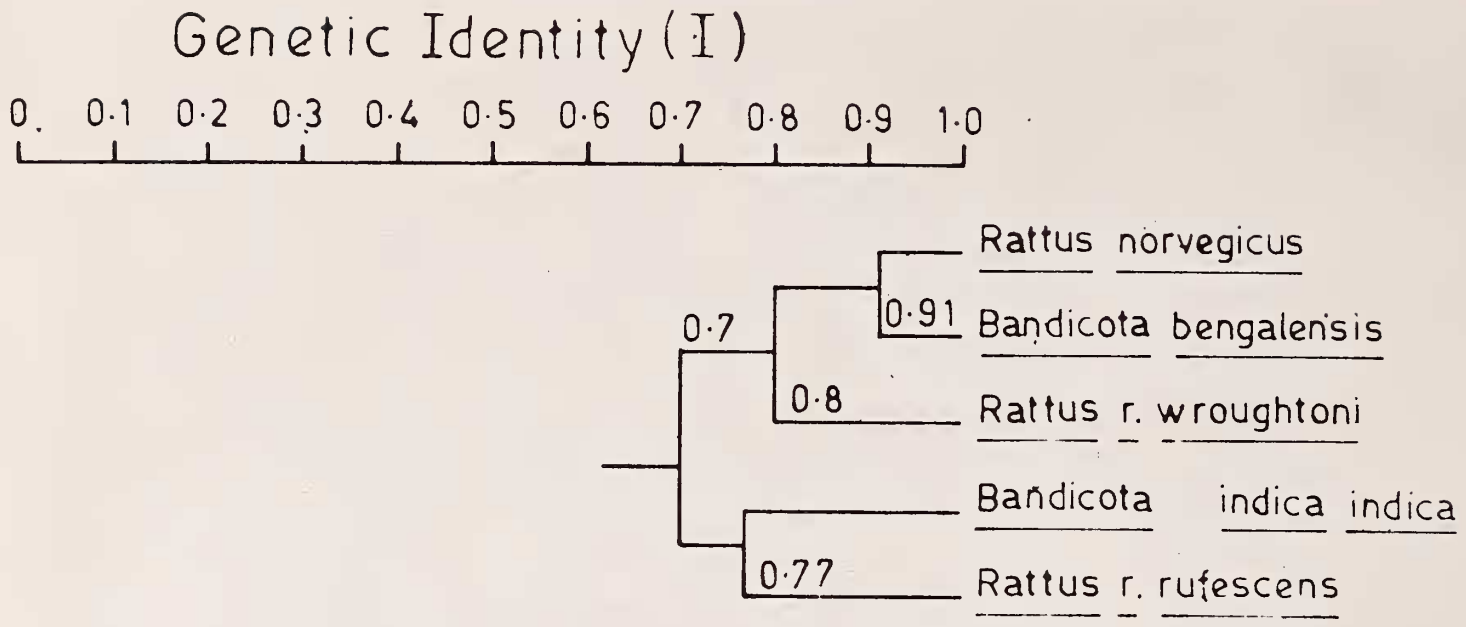

Fig. 2. Dendrogram showing relationships between species generated by cluster analysis

these values of I, the unweighted pair-group method with arithmetic means (UPGMA) of Sneath and Sokal 1973, was applied to construct a dendrogram for these five species (Fig. 2). In terms of genetic identity for haemoglobin locii, all the species studied are rather closely related (I=0.69 to 0.91). However, amongst these, *Bandicota bengalensis* and *Rattus norvegicus* are the closest (I=0.91) followed by *R. rattus wroughtoni* (I=0.8), B. *indica* (0.71) and *Rattus rattus rufescens* (I=0.69). House rat *Rattus rattus rufescens* is closer to *Bandicota indica* than any other species (I=0.77).

The genetic identities of *Bandicota bengalensis* and *Rattus norvegicus* raise certain doubts and speculations. The exotic species *Rattus norvegicus* has a striking similarity, not only in morphology but also in habit and habitats, to the indigenous *B. bengalensis*. Extensive mixing of these two genera has been reported in urban areas like Bombay, Calcutta, Madras, etc. Under these conditions a possibility that these rodents could be interbreeding cannot be ruled out. Such a possibility has already been expressed in rodents possessing white patch (Pradhan and Mithel 1981).

Comparison of *Rattus rattus rufescens* and *R. r. wroughtoni* indicates that these two subspecies of the species *Rattus rattus* have the maximum genetic distance (D=0.48, I=0.62) for the haemoglobin locii. These results when taken together with karyotypic differences reported by

TABLE 2

ESTIMATION OF GENETIC IDENTITY (BELOW DIAGONAL) AND GENETIC DISTANCES (ABOVE DIAGONAL) AMONG MEMBERS OF FIVE SPECIES OF TWO COMMENSAL RODENTS BASED ON NEI, 1972

Serial No.	Names of the species	1	2	3	4	5
1.	*Rattus rattus rufescens* (Gray)	—	0.48	0.27	0.30	0.26
2.	*Rattus rattus wroughtoni* Hinton	0.62	—	0.32	0.17	0.48
3.	*Rattus norvegicus* (Berkenhout)	0.76	0.75	—	0.2	0.29
4.	*Bandicota bengalensis kok (lordi)* (Gray)	0.74	0.84	0.91	—	0.29
5.	*Bandicota indica indica (malabarica)* (Beck.)	0.77	0.62	0.82	0.75	—

Sharma and Raman (1971) in these two subspecies, are in support of the earlier suggestion to elevate *R. rattus rufescens* to a separate species (Tiwari *et al.* 1972). Therefore we feel that, under the present circumstances, a detailed study should be undertaken to confirm the present taxonomic status of *R. rattus rufescens.*

ACKNOWLEDGEMENTS

Our sincere thanks are due to the Director, Zoological Survey of India, Calcutta, the Officer-in-charge, Z.S.I., W.R.S., Pune, and the Principal, R. J. College, Ghatkopar, Bombay, for providing the laboratory and other facilities. We also thank the Municipal Corporations of Greater Bombay and Pune for help in the rodent collection.

REFERENCES

DE SMET & WILLIAM, H.C. (1978): A comparison of the electrophoretic haemoglobin pattern of the vertebrates. *Acta zool. Pathol. Antverp. 70:* 119-131.

DOBZANSKY, T. AYALA, F.J., STEBBINS, G.L. & VALENTINE, J.W. (1976): Evolution. Freeman and Co. Reprinted by Surjeet Publ., Delhi.

ELLERMAN, J.R. (1961): Fauna of India. Mammalia. Vol. III, Part II, First Ed. Manager Publ., Z.S.I., Calcutta.

FERGUSON, A. (1980): Biochemical systematics and evolution. First ed. Blackie, Glasgow and London.

FITCH, W.M. & MORGOLIASH, E. (1970): The usefulness of amino acid and nucleotide sequences in evolutionary studies. *Evol. Biol. 4:* 67-109.

NEI, M. (1972): Genetic Distance between Populations. *Amer. Natur. 106:* 283-292.

PRADHAN, M.S. (1982): A comparison of the electrophoretic haemoglobin pattern of the commensal rodent species. *Proc. of Ind. Acad. Sci. (Anim. Sci) (Bangalore) 91(2):* 159-163.

PRADHAN, M.S. & MITHEL, M. (1981): White patch and its genetic control in some of the Indian Rodent species. *J. Bombay Nat. Hist. Soc. 78(1):* 164-165.

PRADHAN, M.S., BHAGWAT, A.M. & INGALE, S.T. (1985): Haemoglobin polymorphism in commensal species of rodents in India. *In:* Bulletin, Z.S.I. Publ. Calcutta. 7*(i)*: 103-106.

SELANDER, R.K., HUNT, W.G. & YANG, S.Y. (1969): Protein polymorphism and genetic heterozygosity in the two European subspecies of the house mouse. *Evaluation 23(3):* 379-390.

SHARMA, T. & RAMAN, RAJIV (1971): Chromosomes of a few species of rodents of Indian sub-continent. *Mamm. Chrom. Newsl. 12:* 112-115.

SNEATH, P.H.A. & SOKAL, R.R. (1973): Numerical Taxonomy. W.H. Freeman & Co., San Francisco.

TIWARI, K.K., GHOSE, R.K. & CHAKRABORTY, S. (1972); Notes on collection of small mammals from Western Ghats with remarks on the status of *Rattus rufescens* (Gray) and *Bandicota i. malabarica (Shaw). J. Bombay Nat. Hist. Soc. 68(2):* 378-384.

YOSHIDA, T.H., KATO, H., TSUCHIYA, K. & MORIWAKI, K. (1971): Karyotypes and serum transferrin patterns of hybrids between Asian and Oceanian black rats. *Rattus rattus. Chromosoma 34(1):* 40-50.

WRIGHT, C.A. (1974): Biochemical and Immunological Taxonomy of Animals. First Ed. Academic Press, London, N.Y.

ZWEIG, G. & WHITAKER, J.R. (1967): Paper Chromatography and Electrophoresis, Vol. I.A.P.; New York and London.